gene comprising single-stranded regions complementary to the single stranded regions of the construct, are annealed with the construct using ligation-independent cloning to form a targeting construct, such that the positive selection marker is positioned in between the first region and second region of the target sequence, wherein said first and second regions homologously recombine with an endogenous target gene, when said targeting construct is introduced into mouse embryonic stem cells.

52. (New) The construct of claim 51, wherein the single-stranded regions of the construct are non-complementary.

53. (New) The construct of claim 51, wherein the positive selection marker is a neomycin resistance gene.

54. (New) The construct of claim 51, wherein the construct further comprises a screening marker.

55. (New) The construct of claim 54, wherein the screening marker is a fluorescent protein.

56. (New) The construct of claim 51, wherein the construct further comprises a negative selection marker.

57. (New) The construct of claim 56, wherein the negative selection marker is thymidine kinase.

58. (New) A host cell comprising the nucleotide construct of claim 51.

59. (New) A nucleotide construct comprising the sequence set forth in SEQ ID NO:1.

60. (New) A nucleotide construct comprising the sequence set forth in SEQ ID NO:2.

61. (New) A method of producing a targeting construct, the method comprising:
(a) providing a polynucleotide homologous to a target sequence;
(b) generating two fragments of the polynucleotide, the fragments having single-stranded ends which are complementary to a vector having a gene encoding a positive selection marker;
(c) providing the vector having a gene encoding a positive selection marker; and
(d) using ligation independent cloning to insert the two different fragments into the vector to form the construct, wherein the positive selection marker is positioned between the two different fragments in the construct.

62. (New) The method of claim 61, wherein the positive selection marker is a neomycin resistance gene.

63. (New) The method of claim 61, wherein the vector comprises the sequence set forth in SEQ ID NO:1 or the sequence set forth in SEQ ID NO:2.

64. (New) The method of claim 61, wherein the vector comprises a sequence encoding a screening marker.

65. (New) The method of claim 64, wherein the screening marker is a fluorescent protein.

66. (New) The method of claim 61, wherein the vector further comprises a sequence encoding a negative selection marker.

67. (New) The method of claim 66, wherein the negative selection marker is thymidine kinase.

68. (New) The method of claim 61, wherein the polynucleotide sequence of step (a) is obtained by PCR amplifying the fragments with oligonucleotide primers having 5' sequences lacking one type of base and are at least 12 nucleotides in length.

69. (New) The method of claim 68, wherein the oligonucleotide primers are comprised of the sequences set forth in SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5, SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; or SEQ ID NO:10.

70. (New) The method of claim 61, wherein the ligation independent cloning is performed in one step.

71. (New) The method of claim 61, wherein the ligation independent cloning is performed in more than one step.

72. (New) The method of claim 61, wherein the polynucleotide is isolated from a plasmid library.

73. (New) A method of producing a targeting construct, the method comprising:
(a) providing a circular plasmid library;
(b) isolating a polynucleotide sequence from the library using oligonucleotide primers having 5' sequences lacking one type of base, the polynucleotide sequence comprising a first region and a second region of a target sequence;
(c) generating a first fragment comprising the first region and a second fragment comprising the second region;
(d) providing a vector having a gene encoding a positive selection marker; and
(e) inserting the first fragment and second fragment into the vector to form the construct, wherein the positive selection marker is positioned between the first fragment and second fragment in the construct.

74. (New) The method of claim 73, wherein the first and second fragments are inserted using ligation-independent cloning.

75. (New) The method of claim 73, wherein the positive selection maker is a neomycin resistance gene.

76. (New) The method of claim 73, wherein the vector comprises the sequence set forth in SEQ ID NO:1 or the sequence set forth in SEQ ID NO:2.

77. (New) The method of claim 73, wherein the vector comprises a sequence encoding a screening marker.

78. (New) The method of claim 73, wherein the screening marker is a fluorescent protein.

79. (New) The method of claim 73, wherein the vector further comprises a sequence encoding a negative selection marker.

80. (New) The method of claim 73, wherein the negative selection marker is thymidine kinase.

81. (New) The method of claim 73, wherein the oligonucleotide primers are comprised of the sequences set forth in SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; or SEQ ID NO:10.

82. (New) The method of claim 73, wherein the oligonucleotide primers are at least 12 nucleotides in length.